SurfaTech Corporation
Analytical Methods
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ANALYTICAL METHOD

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M-001 -- Appearance

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Scope: This method is applicable to all products.

Summary: The sample is heated to the desired temperature. Once the temperature is reached, the sample is removed from the heat source and observed. A description similar to those of Table I should be reported.

Apparatus:
1. Programmable oven.
2. Steam bath.
3. Thermometer capable of reading °C and °F.

Reagents: None required.

Procedure: Loosen the cap on the sample and place the sample on a steam bath or in an oven set for the desired temperature. Remove the sample occasionally and mix well. Check the temperature of the sample using a thermometer. Once the desired temperature is reached, remove the sample from the steam bath and observe for appearance. Use Table I to assign an appearance.

Calculations: None required.

Precision:

Safety: The heated samples may cause burns. Use caution when handling.

References: None.

Table I

Appearance Classifications

Classification	Description
Sparkling Clear	No visible haze or Tyndall effect in the black box.
Clear	No visible haze in ordinary lighting, but may exhibit a Tyndall effect in the black box.
Slightly Hazy	No visible haze in ordinary lighting but visible in fluorescent lighting.
Hazy	Visible haze in ordinary lighting.
Opaque	Liquid or solid through which one cannot see.

ANALYTICAL METHOD

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M-002 -- Acid Value

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Scope: This method is applicable to animal and vegetable fats and oils, and various products derived from them.

Summary: The acid value is the number of milligrams of potassium hydroxide necessary to neutralize fatty or rosin acids in 1 gram of sample. The sample is weighed into an Erlenmeyer flask, diluted with neutral alcohol, and titrated with 0.1N methanolic potassium hydroxide (KOH) or 0.5N aqueous sodium hydroxide (NaOH), depending on the expected acid value.

If the molecular weight of the fatty acid is known, the free fatty acid content can be calculated using the titration results.

Apparatus:

1. Erlenmeyer flasks, 250 mL.
2. Burette, 10 mL class A.
3. Burette, 50 mL class A.
4. Analytical balance, capable of determining weights to three decimal place accuracy.
5. Stir plate.
6. Stir bars.
7. Steam bath or hot plate.

Reagents:

1. Potassium hydroxide (KOH), 0.1N in methanol (Standardized using LTC-0010).
2. Sodium hydroxide (NaOH), 0.5N in water (Standardized using LTC-0020).
3. Isopropyl alcohol (IPA), reagent grade.
4. Toluene, reagent grade.
5. Chloroform, reagent grade.
6. Phenolphthalein indicator solution, 1.0% in ethanol.

Procedure:

1. Using Table I as a guide, weigh an appropriate amount of sample into a tared Erlenmeyer flask. Record the weight.

2. Add 100 mL of an appropriate neutral alcohol and a few drops of the phenolphthalein indicator solution (**Remark 1**). Place a stir bar in the flask and mix thoroughly to dissolve sample, using heat if necessary.

3. Using Table I as a guide, titrate with the appropriate solution until a faint, pink endpoint appears and persists for 30 seconds. Record the volume of titrant used to reach this endpoint and use Equation 1 in the Calculations section of this method to calculate the acid value.

4. The free fatty acid content can be calculated using Equation 2 in the Calculations section of this method.

5. The acidity (meq/gram) can be calculated using Equation 3 in the Calculations section of this method.

Calculations:

Equation 1

$$\text{Acid Value, mg KOH/gram} = \frac{(\text{mL of titrant})(\text{N of titrant})(56.1)}{(\text{sample wt.})}$$

Equation 2

$$\text{\% Free Fatty Acid} = \frac{(\text{mL of titrant})(\text{N of titrant})(\text{Mwt. of fatty acid})}{(\text{sample wt.})(10)}$$

Where
Mwt. lauric acid = 200
Mwt. palmitic acid = 256
Mwt. oleic acid = 282
Mwt. acetic acid = 60
Mwt. formic acid = 46

Equation 3

$$\text{Acidity, meq/gram} = \frac{(\text{mL of titrant})(\text{N})}{(\text{sample wt.})}$$

Precision: The relative standard deviation for acid value determinations has been determined to be ±0.5% when one sample was analyzed 36 times by different chemists on different days within the same laboratory. This relative standard deviation was determined on a sample with an average acid value of 199.8.

Based on the free fatty acid carbon chain distribution, the theoretical acid value of the sample analyzed was 199.7. Thus, this method reports 100% of the fatty acid present in the sample.

Safety: Isopropyl alcohol is flammable and a dangerous fire risk. Only handle in well ventilated areas.

Chloroform is a known carcinogen. Use in a well ventilated area. Do not get in eyes, on skin, or on clothing.

Toluene is flammable and a dangerous fire risk. Only handle in well ventilated areas.

Potassium hydroxide is corrosive. Do not get dilute solutions in eyes, on skin, or on clothing.

Sodium hydroxide is corrosive. Do not get dilute solutions in eyes, on skin, or on clothing.

Remarks: 1. A solvent system should be chosen which completely dissolves the sample and gives a sharp phenolphthalein endpoint. The three types of solvent systems which can be used are neutralized IPA, chloroform, and neutralized 50:50 IPA/toluene.

References:

1. A.O.C.S. Official Method Cd 3a-63.

Table I

Sample Weight Needed to Obtain a Titration Volume Under 7 mL

Expected Acid Value	Wt. of Sample (±10%),g	Weighing Accuracy, (± grams)	Titrating Solution
0 to 1	20	0.05	0.1N KOH
1 to 4	10	0.02	0.1N KOH
4 to 15	2.5	0.01	0.1N KOH
15 to 75	0.5	0.001	0.1N KOH
75 to 375	0.5	0.001	0.5N NaOH
375 to 1875	0.1	0.0002	0.5N NaOH

ANALYTICAL METHOD

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M-003 -- Base Value

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Scope: This method is applicable to all products which require a phenolphthalein endpoint such as hydroxyl value correction and assaying KOH and NaOH.

Summary: The sample is dissolved in neutralized 3A alcohol and titrated to a phenolphthalein endpoint using a dilute solution of hydrochloric acid. The results are reported internally as "Strong" base value.

Apparatus:
1. Erlenmeyer flasks, 250 mL.
2. Burette, 10 mL class A.
3. Analytical balance, capable of determining weights to three decimal place accuracy.
4. Steam bath or hot plate.
5. Stir plate.
6. Stir bars.

Reagents:
1. Hydrochloric acid (HCl), 0.1N in 3A (Standardized using LTC-0030).
2. Hydrochloric acid (HCl), 0.5N in 3A (Standardized using LTC-0030).
3. 3A alcohol absolute, 95:5:5 ethanol/methanol/IPA, reagent grade (neutralized to first phenolphthalein endpoint).
4. Phenolphthalein indicator solution, 1.0% in ethanol.

Procedure:

1. Using Table I as a guide, weigh an appropriate amount of sample into a tared Erlenmeyer flask. Record the weight.

2. Add about 75 mL of neutralized 3A alcohol and a few drops of phenolphthalein indicator solution. Place a stir bar in the flask and mix thoroughly to dissolve sample, using heat if necessary. Allow the sample solution to cool to room temperature before titrating.

3. Titrate with the appropriate HCl solution (See Table I) until the pink color disappears from the sample solution. Record the volume of titrant used to reach this endpoint. Using Equation 1 in the Calculations section of this method, determine the amine value. Report this value to one decimal place.

Calculations: Equation 1

$$\text{Base Value} = \frac{(\text{mL of titrant})(\text{N of titrant})(56.1)}{(\text{sample wt.})}$$

Safety: The samples are basic in nature and therefore corrosive. Caution should be used when handling. Do not get in eyes, on skin, or on clothing.

Hydrochloric acid can burn skin. Do not get in eyes, on skin, or on clothing.

3A alcohol is flammable and a dangerous fire risk. Only handle in well ventilated areas.

Table I

Sample Weight Needed to Obtain a Titration Volume Under 7 mL

Expected Base Value	Wt. of Sample (+10%), g	Titrating Solution
0 to 1	20	0.1 N HCl
1 to 4	10	0.1 N HCl
4 to 15	2.5	0.1 N HCl
15 to 75	0.5	0.1 N HCl
75 to 375	0.5	0.5 N HCl
375 to 1875	0.1	0.5 N HCl

ANALYTICAL METHOD

M-004 -- Color, Gardner

Scope: This method is applicable to products in the liquid or solid state which do not differ in hue appreciably from the standards.

Summary: This method will assign a number, between 1- and 18+, which corresponds to the color of the sample as compared to a set of 18 standards. A Gardner Color may be reported on a product which differs in hue from the standards. This color will be reported as the resulting color plus the designation "Off Hue".

Apparatus:
1. 18 glass standards, 1963 series.
2. Gardner-Delta Color Comparator.
3. Comparison tubes.
4. Funnel.
5. Filter paper, Ahlstrom #505.
6. Ring stand.

Reagents: None required.

Procedure:

1. Melt the sample if it is not in a liquid state. Inspect the sample for any foreign matter and filter the sample if any is present.

2. Mix the sample thoroughly and pour into a comparison tube. Place the comparison tube in the comparator and compare with the standards to determine which standard is nearest in color to the sample.

3. Report the color of the sample as the number of the standard most closely matching the sample. If the sample falls between two standards, it will be reported as "+" or "-" (depending on whether it is darker or lighter than the standard it most closely resembles. Thus, between colors 5 and 6, the steps will be 5, 5+, 6-, and 6. If the color is lighter than 1, it will be reported as "1-". If the color is darker than 18, it will be reported as "18+".

Calculations: None required.

Precision: The color should not vary more than 1/3 unit from chemist to chemist. Off-hue products may vary up to 3 units.

Safety: The molten product is hot and may cause thermal burns. Use caution when handling.

References: A.O.C.S. Official Method Td 1a-64.

ANALYTICAL METHOD

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M-005 -- Hydroxyl Value by Acetylation

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Scope: This method is applicable to any nonionic product which has primary hydroxyl values.

Summary: The hydroxyl value is the number of milligrams of potassium hydroxide equivalent to the hydroxyl content of one gram of sample. The sample is weighed into an Erlenmeyer flask and diluted with 20 mL of acetylating reagent. This mixture is refluxed for 30 minutes and titrated to a phenolphthalein endpoint with 2.0N sodium hydroxide.

Apparatus:

1. Erlenmeyer flasks, 250 mL with ground glass joints.
2. Burette, 100 mL class A with 0.1 mL divisions.
3. Analytical balance, capable of determining weights to three decimal places.
4. Stir plate.
5. Stir bars.
6. Reflux condensers with ground glass joints.
7. Pipette, 20 mL class A volumetric.
8. Graduated cylinder, 25 mL.
9. Glass bottle, >500 mL.

Reagents:

1. Sodium hydroxide (NaOH), 2.0N (Standardized using LTC-0010).
2. Pyridine, reagent grade.
3. Acetic anhydride, reagent grade.
4. Phenolphthalein indicator solution, 1.0% in ethanol.

Procedure:

Preparation of Acetylating Reagent:

1. In a glass bottle, add 1.4 mL deionized water and 400 mL pyridine. Mix thoroughly.

2. Add 50 mL acetic anhydride to the solution and mix thoroughly again.

Hydroxyl Value Analysis:

1. Every sample should be analyzed in duplicate. Use Equation 1 in the Calculations section of this method to determine the appropriate sample size (**Remark 1**). Weigh this calculated amount into a tared Erlenmeyer flask. Record the weight.

2. Pipette 20 mL of the acetylating reagent into each of the flasks containing sample as well as two Erlenmeyer flasks which will act as blanks. Add boiling stones to the samples and blanks.

3. Place all of the flasks on hot plates and connect to reflux condensers (**Remark 2**). Reflux for 30 minutes.

4. After refluxing is complete, wash down each condenser with about 10 mL of deionized water and catch the rinsing in the respective Erlenmeyer flasks.

5. Remove the flasks from the condensers and allow them to cool.

6. Add a few drops of phenolphthalein indicator solution and a stir bar to each flask. Titrate each blank and sample with 2.0 N NaOH to a faint, pink endpoint. Record the respective titration volumes and use Equation 2 in the Calculations section of this method to determine the uncorrected hydroxyl value of each sample. The corrected hydroxyl value can be determined using Equations 3 or 4, whichever is appropriate.

7. Equations 5, 6, and 7 can be used for determining the calculated hydroxyl value, average molecular weight, and % residual alcohol.

Calculations:

Equation 1

$$\text{Appropriate Sample Size} = \frac{(2.5)(2.0)(56.1)}{\text{(expected OHV)}}$$

Equation 2

$$\text{Uncorrected OHV} = \frac{(\text{mL Blank} - \text{mL Sample})(N)(56.1)}{\text{sample wt.}}$$

Where Blank = average of two blank runs

Equation 3

Corrected OHV = Uncorrected OHV + Acid Value (from LTC-1010)

Equation 4

Corrected OHV = Uncorrected OHV - Base Value (from LTC-1020)

Equation 5

$$\text{Calculated OHV} = \frac{56100}{\text{Mwt of product}} \times \#\text{ of OH groups}$$

Equation 6

$$\text{Average Mwt} = \frac{56100}{\text{OHV}} \times \#\text{ of OH groups}$$

Equation 7

$$\text{Residual Alcohol, \%} = \frac{\text{OHV}}{\text{Mwt of product}} \times 100$$

Precision: The relative standard deviation for hydroxyl value determinations has been determined to be ±1.1% when one sample was analyzed 36 times by different chemists on different days within the same laboratory. This relative standard deviation was determined on a sample with an average (uncorrected) hydroxyl value of 278.6.

The corrected hydroxyl value of this sample was 278.7 and theoretical hydroxyl value of 100% pure tridecyl alcohol is 280.5. Therefore, this method reports at least 99.3% of the hydroxyl bearing molecules present in a sample.

Safety: Pyridine is flammable and toxic. Avoid breathing in fumes. Handle in well ventilated areas at all times. Do not get in eyes, on skin, or on clothing.

Acetic anhydride can cause burns and irritate eyes. Avoid breathing in fumes. Handle in well ventilated areas at all times. Do not get in eyes, on skin, or on clothing.

Sodium hydroxide is corrosive. Do not get dilute solutions in eyes, on skin, or on clothing.

Remarks: 1. The ideal titration volume of the sample is about 3/4 the titration volume of the blank. This calculation will give these titration volumes.

2. Before condensing begins, verify that there is cold water passing through the condensers. This will aid in the condensing of the samples.

References:

1. A.O.C.S. Official Method Cd 13-60.

ANALYTICAL METHOD

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M-006 -- Saponification Value

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Scope: This method is applicable to all fats and oils, as well as products derived from them such as esters and fatty acids.

Summary: The saponification value is the amount of alkali necessary to saponify a definite quantity of the sample. It is expressed as the number of milligrams of potassium hydroxide (KOH) required to saponify one gram of the sample.

A sample is refluxed in 0.5N methanolic KOH for 1.5 hours and titrated using 0.5N HCl.

Apparatus:

1. Erlenmeyer flasks, 250 or 300 mL with ground glass joints.
2. Liebig condensers, with ground glass joints.
3. Pipettes, 20 mL class A volumetric.
4. Burette, 50 mL class A with 0.2 mL divisions.
5. Stir bars.
6. Stir plate.
7. Hot plate.
8. Analytical balance, capable of determining weights to three decimal place accuracy.
9. Syringes, 3 and 5 mL.
10. Graduated cylinder, 25 mL.
11. Boiling stones.

Reagents:

1. Potassium hydroxide (KOH), ethanolic 0.5N (Prepared using LTC-0010), (**Remark 1)**.
2. Hydrochloric acid (HCl), 0.5N (Standardized using LTC-0030).
3. Phenolphthalein indicator solution, 0.1% in ethanol.

Procedure:

1. Melt the sample, if not a liquid, and mix thoroughly to ensure homogeneity. Using Table 1 as a guide, weigh the appropriate amount of sample into an Erlenmeyer flask (**Remark 2**). Record the weight.

2. Pipette 50 mL of 0.5N KOH into the flask, add some boiling stones, and reflux for 1.5 hours. Make sure that there is cold water going through the condensers so as to aid in the condensing of the sample back into the Erlenmeyer flasks.

3. Prepare and run a blank simultaneously with the samples by pipetting 50 ml of 0.5N KOH into an empty flask, adding some boiling stones, and refluxing along side the samples (**Remark 1**).

4. After 1.5 hours of refluxing, rinse the inside of the condensers with about 25 mL of deionized water and catch the rinsings in the Erlenmeyer flasks. Remove the flasks from the condensers and allow the sample solutions to cool to room temperature.

5. To each flask, add 3 to 5 drops of phenolphthalein indicator and a stir bar. Titrate, while mixing, with 0.5N HCl until the pink color just disappears. Record the respective titration volumes used to reach each endpoint.

6. Using Equation 1 in the Calculations section of this method, calculate the SAP value of the samples analyzed. Report the results to one decimal place.

7. The ester value of a product can be determined using Equation 2, if the acid value is also known.

Calculations: Equation 1

$$\text{SAP value} = \frac{(\text{mL Blank} - \text{mL Sample})(\text{N of HCl})(56.1)}{(\text{wt. of sample})}$$

Equation 2

Ester value = Saponification value – Acid value

Precision: The relative standard deviation for saponification value determinations has been determined to be ±0.5% when one sample was analyzed 36 times by different chemists on different days within the same laboratory. This relative standard deviation was determined on a sample with an average saponification value of 336.0.

Using the free fatty acid carbon chain distribution of this sample, the theoretical saponification value was determined to be 336.7. Therefore, this method reports approximately 99.8% of the theoretical saponification value.

Safety: Potassium hydroxide is corrosive and can burn skin. Do not get in eyes, on skin, or on clothes.

Hydrochloric acid can burn skin. Do not get in eyes, on skin, or on clothes.

Remarks: 1. The 1.0N KOH solution is usable for at least 3 months provided the solution is protected from carbon dioxide and blanks are determined with each analysis.

2. The flask must be completely clean and completely dry before using.

References: A.O.C.S. Official Method Cd 3c-91.

Table 1

SAP Value Expected	Sample Wt. (grams)	SAP Value Expected	Sample Wt. (grams)
0 to 59	10.0 to 12.0	180 to 199	3.3 to 4.1
60 to 79	9.0 to 11.0	200 to 219	3.0 to 3.7
80 to 99	7.0 to 8.6	220 to 239	2.7 to 3.4
100 to 119	5.7 to 7.0	240 to 259	2.5 to 3.1
120 to 139	4.9 to 5.9	260 to 279	2.2 to 2.7
140 to 159	4.2 to 5.1	280 to 300	2.2 to 2.7
160 to 179	3.9 to 4.8		

ANALYTICAL METHOD

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M-007-- Iodine Value (Wijs Method)

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Scope: This method is applicable to all normal fatty acids, oils and fatty amines which do not contain conjugated double bonds. It cannot be used for quaternary ammonium compounds.

When iodine value is determined on fatty acids containing conjugated double bonds, the result is not to be used as a value of total unsaturation, but rather a value to compare with similar systems' degree of unsaturation (**Remark 1**).

Summary: The Iodine Value is a measure of the unsaturation of fatty acids and is expressed in terms of the number of centigrams of iodine absorbed per gram of sample (percent iodine absorbed).

A sample is dissolved in chloroform and then reacted, in the dark, with Wijs solution for a set amount of time. KI and deionized water are added to the flask and the solution is titrated with 0.1N sodium thiosulfate.

Apparatus:

1. Erlenmeyer flasks, 250 mL Iodine Determination with ground glass stoppers.
2. Analytical balance, capable of determining weights to four decimal places.
3. Pipette, 10 & 25 mL class A volumetric.
4. Burette, 50 mL class A with 0.2 mL divisions.
5. Graduated cylinders, 50 mL.
6. Stir bars.
7. Stir plate.
8. Steam bath.

Reagents:

1. Chloroform, reagent grade.
2. Wijs solution, reagent grade (**Remark 2**).
3. Potassium iodide (KI) solution, 15% in deionized water.
4. Mercuric acetate solution, 2.5% in acetic acid.
5. Sodium thiosulfate, 0.1N (Standardized using LTC-0050).
6. Starch indicator solution, 1% in deionized water (**Remark 3**).

Procedure:

1. Using Table I as a guide, weigh an appropriate amount of sample into a tared Erlenmeyer flask (**Remarks 4 & 5**). Record the weight. Label the flask accordingly.

2. Dissolve the sample by adding 25 mL of chloroform to the flask and swirling the flask. If needed, heat the flask on a steam bath to completely dissolve the sample.

3. After the sample solution has cooled to room temperature, pipette 25 mL of Wijs solution into the flask and swirl the contents till thoroughly mixed (**Remark 2 & 6**).

4. Prepare a blank sample by pipetting 25 mL of chloroform and 25 mL of Wijs solution into an empty Erlenmeyer flask (**Remark 4**). Label the flask accordingly.

5. Stopper the flasks with a ground glass stopper. Pipette about 5 mL of the 15% KI solution into the stopper well.

6. Using a timer, store the flasks in a dark place for 30 minutes (60 minutes for expected iodine values greater than 150) to allow the reaction to take place completely (**Remark 7**).

7. After the reaction is complete, remove all of the flasks from the dark at the same time. Add about 20 mL of the 15% KI solution and 75 mL of deionized water to each of the flasks. Add a stir bar to each flask and mix well.

8. Using 0.1N sodium thiosulfate, titrate the blank sample to a pale yellow endpoint (**Remark 8**). Add about 2 mL of the starch indicator solution to the flask and continue titrating until the blue color just disappears (usually a white endpoint). Repeat this titration for each of the samples.

9. Using Equation 1 in the Calculations section of this method, calculate the iodine value. Report this value to 1 decimal place.

Calculations:

Equation 1

$$\text{Iodine Value (IV)} = \frac{(\text{mL Blank} - \text{mL Sample})(N)(12.69)}{\text{sample wt.}}$$

Precision: The relative standard deviation for iodine value determinations has been determined to be ±1.3% when one sample was analyzed 36 times by different chemists on different days within the same laboratory. This relative standard deviation was determined on a sample with an average iodine value of 134.7.

Safety: Chloroform is a known carcinogen. Do not breathe in vapors. Use in a well ventilated area at all times. Do not get in eyes, on skin, or on clothing.

Wijs solution causes severe burns, and the vapors can cause lung and eye damage. Use in a well ventilated area at all times. Do not get in eyes, on skin, or on clothing.

Acetic acid is corrosive and toxic. Use caution when handling. Do not get in eyes, on skin, or on clothing.

Mercuric acetate is corrosive and highly toxic. Use caution when handling. Do not get in eyes, on skin, or on clothing.

Remarks:

1. This is due to the fact that addition to one double bond of a conjugated diene and two double bonds of a conjugated triene goes rapidly but saturation of the remaining double bond is extremely slow.

2. Because the preparation of the Wijs solution is time consuming and involves the use of both hazardous and toxic chemicals, this solution may be purchased from a chemical supplier. Only use solutions which contain no carbon tetrachloride. Store in a explosion proof refrigerator to keep the solution cool and out of the light. Never allow the temperature of the solution to rise above 25-30°C. All Wijs solutions are sensitive to temperature, moisture and light.

3. The 1% starch solution can be purchased from a chemical supplier. However, if it is to be made in the lab, "Potato Starch for Iodometry" is recommended because it produces a deep blue color in the presence of the iodonium ion. "Soluble Starch" is not recommended because a consistent deep blue color may not be developed when some soluble starches interact with the iodonium ion. The following are suitable starches: Soluble Starch for Iodometry, Fisher S516-100; Soluble Potato Starch, Sigma S-2630; Soluble Potato Starch for Iodometry, J.T. Baker 4006-04.

4. All glassware must be completely clean and completely dry!

5. When analyzing dehydrated castor oil fatty acids or it's derivatives, weigh 0.11 - 0.13 grams of sample. Due to the amount of free hydroxyl groups in castor oil, it yields high iodine values.

6. When analyzing fatty amines, add 10 mL of 2.5% mercuric acetate solution along with the Wijs solution. Add 10 mL of this solution to the blank as well. The reaction time is only 3 minutes for fatty amines.

7. If the reaction is not terminated within 3 minutes of the designated reaction time (30 or 60 minutes), the sample must be discarded and reanalyzed.

8. The samples must be titrated within 30 minutes of the reaction completion (when they were removed from the dark). Otherwise, the samples must be discarded and reanalyzed.

References:

1. A.O.C.S. Official Method Cd 1-25.
2. A.O.C.S. Official Method Tg 1a-64.
3. A.O.C.S. Official Method Tg 2a-64.
4. Iodine Value Wijs Method, Hodag Co., Semega, Jayne L., 1978.

ANALYTICAL METHOD

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M-008 -- Cloud Point of Nonionic Surfactants

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Scope: This method is applicable to all nonionic surfactants.

Summary: The cloud point is that temperature at which, under the conditions of this test, the sample loses solubility and an emulsion appears.

A sample solution is cooled to below the cloud point (if necessary) and then heated at a rate of 1-2°C per minute. The cloud point is reached when the thermometer can no longer be seen through the side of the bottle. The cloud point is reported to the nearest 0.1°C.

Apparatus:
1. Thermometer capable of measuring between 0°C and 100°C, with 0.1°C divisions.
2. Beaker, 150 mL.
3. Stir bars.
4. Stir/hot plate.

Reagents:
1. Sodium chloride solution, 10% in deionized water.

Procedure:

1. Prepare 100 mL of the appropriate sample solution in a 150 mL beaker. Add a stir bar and mix well.

2. Cool the sample solution in an ice water bath till it is clear (**Remark 1**). Stir the cooling sample with a thermometer at a rate sufficient to keep the temperature throughout the sample uniform.

3. Suspend the thermometer in the solution with the bulb 1/2 inch from the bottom of the beaker (**Remark 2**).

4. With mild stirring, heat the beaker at a rate of 1-2°C per minute. The cloud point is that temperature at which the immersed portion of the thermometer is no longer visible when viewed horizontally through the bottle and sample. Report the cloud point to the nearest 0.1°C.

Calculations: None required.

Precision:

Safety: The hot plate and sample may be hot and can cause thermal burns. Use caution when handling.

Remarks:

1. It is important that the sample solution is cooled till it is clear (or completely solublized). If the sample solution is not clear, inaccurate cloud point values will be obtained.

2. Do not remove the thermometer from the sample, since to do so may introduce air bubbles which will interfere with the test.

References:
1. A.O.C.S. Official Method Cc 6-25, Reapproved 1989.

ANALYTICAL METHOD

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M-009-- Cloud Point, °C

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Scope: This method is applicable to all normal animal and vegetable fats and oils.

Summary: The cloud point is that temperature at which, under the conditions of this test, a cloud is induced in the sample caused by the first stage of crystallization.

A liquid sample is cooled in a ice water bath while constantly being stirred with a thermometer. The cloud point is reached when the thermometer can no longer be seen through the side of the bottle. The cloud point is reported in degrees Celsius.

Apparatus:
1. Water bath prepared with water, ice and salt (temperature > 2°C).
2. Bottle, 4 ounce.
3. Thermometer capable of measuring between -2°C and 68°C, with 0.1°C divisions.

Reagents: 1. None required.

Procedure: 1. This test is performed on samples which are in a liquid form. If the sample is not a liquid, heat about 75 grams of sample to 130°C.

2. Pour about 45 grams of the sample into a 4 ounce bottle and begin cooling in an ice water bath (**Remark 1**). Stir the cooling sample with a thermometer at a rate sufficient to keep the temperature throughout the sample uniform.

3. When the sample has reached a temperature which is about 10°C above the expected cloud point, begin stirring steadily and rapidly in a circular motion so as to prevent super cooling and solidification of fat crystals on the sides or bottom of the bottle (**Remark 2**).

4. Remove the bottle from the bath and inspect regularly. The cloud point is that temperature at which the immersed portion of the thermometer is no longer visible when viewed horizontally through the bottle and sample. Report the cloud point to the nearest degree Celsius.

Calculations: None required.

Precision:

Safety: The thermometers contain mercury. Mercury is a poisonous and highly toxic material. Use caution when using thermometers and if a thermometer should break, use a mercury spill clean-up kit to properly clean up the mercury.

Remarks: 1. The bottle should be submerged in the ice water bath so that the sample level in the bottle is even with the water level of the bath.

2. Do not remove the thermometer from the sample, since to do so may introduce air bubbles which will interfere with the test.

References: A.O.C.S. Official Method Cc 6-25, Reapproved 1989.

ANALYTICAL METHOD

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M-010 -- % Solids

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Scope: This method is applicable to all products.

Summary: A sample is weighed into two separate aluminum dishes and exposed to 105°C temperatures for two hours. The % solids or % volatiles is then determined based on the residue in the aluminum dishes.

Apparatus:
1. Analytical balance, capable of determining weights to three decimal places.
2. Oven, capable of maintaining a temperature of 105°C.
3. Thermometer ,capable of measuring 105°C.
4. Aluminum weighing dishes.
5. Dessicator capable of maintaining a moisture free environment.

Reagents: None required.

Procedure:

1. Weigh two aluminum dishes. Record the weights respectively as TARE.

2. Weigh about 2 grams of sample into each aluminum dish. Record the sample weights respectively as INITIAL.

3. Spread the sample evenly across the entire surface of each aluminum dish.

4. Place the aluminum dishes in a 105°C oven for two hours.

5. After two hours, remove the aluminum dishes from the oven and cool to room temperature in a dessicator.

6. Weigh the aluminum dishes. Record the weights respectively as FINAL.

7. Using Equation 1 in the Calculations section of this method, determine the % Solids of each aluminum dish (Equation 2 can be used to determine % volatiles).

8. Repeat Steps 4 through 7, heating for 30 minutes rather than two hours, until the % Solids of each aluminum dish does not change by more than 0.1%.

Calculations:

Equation 1

$$\% \text{ Solids} = \frac{\text{FINAL} - \text{TARE}}{\text{INITIAL} - \text{TARE}} \times 100$$

Where: FINAL = Final wt. of the residue and aluminum dish
TARE = Tare wt. of the aluminum dish
INITIAL = Initial wt. of the sample and aluminum dish

Equation 2

$$\% \text{ Volatiles} = 100 - \% \text{ Solids}$$

Safety: The oven and sample may be hot and cause thermal burns. Use caution when handling.

ANALYTICAL METHOD

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M-011-- Viscosity by Brookfield Viscometer

==========

Scope: This method is applicable to non-thixotropic liquid samples.

Summary: The sample is heated to the desired temperature and analyzed for viscosity using a Brookfield Viscometer. The sample must be relatively free of entrained air bubbles for the instrument to function properly.

Apparatus:
1. Brookfield Viscometer, Model RVF (or equivalent).
2. Steam bath.
3. Programmable oven.
4. Thermometer capable of °C and °F.
5. Beakers, varying sizes. .

Reagents: None required.

Procedure: The attached diagram can be used to aid in understanding how the instrument is operated.

1. Select the proper spindle (A). Transfer the sample to a beaker large enough to hold the viscometer spindle. Place the beaker on a steam bath or in an oven set for the desired temperature. Remove the sample occasionally and mix well. Check the temperature of the sample using a thermometer. Once the desired temperature is reached, remove the sample from the heat source and check viscosity.

2. Attach the spindle (A) to the upper coupling (B) by holding the coupling between the thumb and forefinger while cautiously rotating the spindle counterclockwise. Avoid undue side pressure.

3. Set the knob (H) to the minimum speed which includes the centipoise range on the material to be tested. The uppermost number on the knob indicates the revolutions per minute (rpm).

4. Immerse the spindle into the sample up to the middle of the indentation in the shaft (E).

5. Turn the viscometer on and allow it to run until a constant reading (usually 5 to 10 revolutions). Using Equation 1 in the Calculations section of this method, determine the viscosity of the sample. Report the viscosity to one decimal place accuracy for viscosities under 100. For viscosities over 100, report the viscosity to the nearest whole number.

Calculations: Equation 1

Viscosity = (Reading obtained) x (Factor for the spindle/speed comb.)

Precision:

Safety: The heated samples may cause burns. Use caution when handling.

Use caution when operating the Brookfield viscometer. Dangling objects may get caught in the spindle and cause injury.

References: 1. "More Solutions to Sticky Problems", Brookfield Engineering Laboratories, Inc., 1992.

ANALYTICAL METHOD

==========

M-011-- Viscosity by Brookfield Viscometer

==========

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Apparatus:
1. Brookfield Viscometer, Model RVF (or equivalent).
2. Steam bath.
3. Programmable oven.
4. Thermometer capable of °C and °F.
5. Beakers, varying sizes. .

Reagents: None required.

Procedure: The attached diagram can be used to aid in understanding how the instrument is operated.

1. Select the proper spindle (A). Transfer the sample to a beaker large enough to hold the viscometer spindle. Place the beaker on a steam bath or in an oven set for the desired temperature. Remove the sample occasionally and mix well. Check the temperature of the sample using a thermometer. Once the desired temperature is reached, remove the sample from the heat source and check viscosity.

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Calculations: Equation 1

Viscosity = (Reading obtained) x(Factor for the spindle/speed comb.)

Precision:

Safety: The heated samples may cause burns. Use caution when handling.

Use caution when operating the Brookfield viscometer. Dangling objects may get caught in the spindle and cause injury.

References: 1. "More Solutions to Sticky Problems", Brookfield Engineering Laboratories, Inc., 1992.

ANALYTICAL METHOD
==========M-012-- Titer, °C==========

Scope: This method is applicable to fatty acids.

Summary: This method determines the solidification point or “titer” of fatty acids.

Apparatus: 1. Titer stirring assembly consisting of:
   a. Water bath (2000 mL beaker).
   b. Wide mouth bottle.
   c. Test tube, 25x100 mm.
   d. Thermometer.
   e. Wire stirrer with one end bent into a loop.
   f. Corks, six.

Reagents: None required.

Procedure: 1. Add water to the designated level of the bath.

2. Adjust water bath temperature to 15-20°C below the expected titer point.

3. Melt sample (if solid) not greater than ~15°C above expected titer.

4. Pour the sample into the test tube to he immersion mark of the thermometer.

5. Place the thermometer and wire stirrer into the test tube keeping them equidistant from the sides.

6. Place test tube assembly into wide mouth bottle.

7. The agitation with the wire stirrer is started while the temperature of sample is 10°C above the titer point.

8. Stir sample at a rate of 20 strokes per minute.

9. Record temperature of sample every minute.

10. Stir until the temperature remains constant for 30 seconds or it begins to rise.

11. Discontinue stirring immediately. Observe and record the increase in temperature.

12. The titer point is the highest temp reached by the thermometer during this rise.

Calculations: None required.

Precision: Not determined.

References: 1. A.O.C.S. Official Method Da 13-48.

ANALYTICAL METHOD

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M-013 -- Unsaponifiable Matter

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Scope: This method is applicable to products containing mineral oil, wax, or fatty alcohol.

Summary: This method determines the amount of matter soluble in fats and oils which cannot be saponified by caustic alkali.

Apparatus:

1. Erlenmeyer flask w/ ground glass joint, 250 mL.
2. Hot plate.
3. Condenser with cold water running through.
4. Separatory funnel, 250 mL.
5. Stokes flask w/ stopper and tubing, 250 mL.
6. Analytical balance, capable of measuring to three decimal place accuracy.
7. Dessicator capable of maintaining moisture-free environment.
8. Forced air oven capable of maintaining 105°C temperature.
9. Thermometer capable of measuring 105°C temperature.
10. Pipette bulb.
11. Pipette, 50 mL class A.

Reagents:

1. Reagent alcohol, ACS reagent grade diluted to 10% with deionized water.
2. Saponification reagent, prepared using LTC-1150.
3. Petroleum ether, ACS reagent grade.

Procedure:

1. Weigh five grams of sample into a 250 mL Erlenmeyer flask with ground glass joint. Record the weight to three decimal places.

2. Pipette 50 mL of saponification reagent into the flask.

3. Place the flask on a hot plate and connect a condenser to it with cold water running through it. Allow the solution to reflux for one hour.

4. After one hour, quantitatively rinse the inside of the condenser with about 20 mL of deionized water.

5. Remove the flask from the hot plate and quantitatively transfer the solution to a stokes flask using deionized water.

6. Add enough water to the stokes flask to bring the fluid level to the neck of the flask (just below the bulb of the flask).

7. Add about 50 mL of petroleum ether to the stokes flask. Stopper the flask and shake gently for one minute.

8. Using the stopper and glass tubing assembly, transfer the petroleum ether layer to a 500 mL separatory funnel.

9. Repeat Steps 7 and 8 until a total of five extractions are performed. Keep adding the petroleum ether layer to the same separatory funnel.

10. Add about 25 mL of the 10% reagent alcohol solution to the separatory funnel. Stopper the separatory funnel and shake gently for one minute.

11. Dispose of the 10% reagent alcohol layer.

12. Repeat Steps 10 and 11 until a total of three washes are performed of the petroleum ether layer.

13. Transfer the washed petroleum ether layer to a tared 250 mL beaker. Place the beaker on a steam bath and evaporate the petroleum ether to dryness.

14. Place the beaker in a 105°C oven for approximately 10 minutes (or until a constant weight is achieved).

15. Weigh the beaker and determine the weight of the residue to three decimal place accuracy.

16. Using Equation 1 in the Calculations section of this method, calculate the percent unsaponifiable matter in the sample. Report the results to two decimal place accuracy.

Calculations: Equation 1

$$\% \text{ Unsaponifiable Matter} = \frac{\text{Wt. of residue}}{\text{Sample wt.}} \times 100$$

Precision:

Safety: Petroleum ether is extremely flammable. Use in a well ventilated area and keep ignition sources away.

Reagent alcohol is flammable and a dangerous fire risk. Only handle in well ventilated areas.

Saponification reagent is corrosive. Do not get in eyes, on skin, or on clothing.

Methanol is flammable and toxic. Use in well ventilated areas and avoid getting in eyes, on skin, or on clothing.

Remarks:

References:

1. A.O.C.S. Official Method Tk 1a-64, reapproved 1989.

ANALYTICAL METHOD

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M-014 -- Monoester, Diester and Free Phosphoric Acid Determination

==========

Scope: This method is applicable to all phosphate esters.

Summary: This method can be used to determine the three acid values for phosphate esters as well as the amount of monoester, diester, and free phosphoric acid in the sample.

A phosphate ester sample is titrated with 0.1N methanolic KOH to three endpoints. The first endpoint represents the amount of KOH needed to neutralize one H+ of the phosphoric acid, one H+ of the monoester, and the H+ of the diester. The second endpoint represents the amount of KOH needed to neutralize a second H+ of the phosphoric acid and the second H+ of the the monoester. The third endpoint represents the amount of KOH needed to neutralize the third H+ of the phosphoric acid (See Figure I for the chemistry involved).

Apparatus:

1. Analytical balance, capable of determining weights to three decimal places.
2. Brinkmann 716 Titrino (or equivalent) with a glass combination electrode.
3. Beakers, 250 mL.
4. Stir bars.
5. Stir plate.
6. Hot plate.
7. Syringe, 5cc.
8. Kimwipes.

Reagents:

1. Potassium hydroxide (KOH), 0.1N in methanol (Standardized using LTC-0010).
2. Isopropyl alcohol (IPA), reagent grade, neutralized to phenolphthalein endpoint.
3. Saturated calcium chloride solution, prepared using neutralized deionized water.
4. Deionized water, neutralized to pH 7.
5. Phenolphthalein indicator solution, 1.0% in ethanol.
6. Bromocresol green indicator solution, 1.0% in ethanol.

Procedure:

Instrument Set-up:

1. Program the autotitrator to contain the two sets of parameters outlined in Figures II and III of this method. Modify common variable C31, in the Configuration, to reflect the normality of the KOH solution.

2. Verify that the electrode contains 3N KCl in the inner-cell. Fill if necessary.

3. Uncap the electrode and clean with IPA. Dry with a Kimwipe.

4. Remove any air bubbles from the dispensor tip of the exchange unit.

Potentiometric Titration to Inflection Endpoints (**Remark 1**):

1. Using Equation 1 in the Calculations section of this method, determine the appropriate sample size. Weigh this calculated amount into a 250 mL beaker. Record the weight and label the beaker as Sample #1.

2. Weigh the calculated amount into a second 250 mL beaker. Record the weight and label the beaker as Sample #2.

3. Add about 100 mL of the appropriate solvent (See Table I) and a stir bar to each beaker. Stir each sample solution at medium speed until the sample is completely dissolved, using heat if necessary.

4. Titrate Sample #1 with 0.1N methanolic KOH using the parameters outlined in Figure II. Continue titrating until two titration endpoints are observed and the pH is greater than 12 (**Remark 2**). Record the pH of EP2.

5. Modify the titration parameters to stop at the pH of the second endpoint (EP2). The parameter which is to be changed is specified in Figure II of this method.

6. Titrate Sample #2 with 0.1N methanolic KOH, using the modified parameters of Figure II, until the specified pH is reached (the instrument will automatically stop at the specified pH). Record AV-1.

7. Add 2 drops of saturated calcium chloride to the sample solution (**Remark 3**).

8. Titrate with 0.1N methanolic KOH, using the parameters outlined in Figure III, until an endpoint (the third endpoint) is observed and the pH is greater than 12 (**Remark 2**). Record AV-2 and AV-3.

9. Using Equations 2, 3 and 4 in the Calculations section of this method, determine AV-1, AV-2 and AV-3 (if necessary). Using Equations 5, 6 and 7 in the Calculations section of this method, determine the Monoester, Diester and Free Phosphoric Acid content.

Manual Titration to Colorimetric Endpoints (**Remark 1**):

1. Using Equation 1 in the Calculations section of this method, determine the appropriate sample size. Weigh two times this calculated amount into two 250 mL beakers and label as Sample #1 and Sample #2. Record the respective weights.

2. Add about 100 mL of the appropriate solvent (See Table I) and a stir bar to each beaker. Stir each sample solution at medium speed until the sample is completely dissolved, using heat if necessary.

3. Add a few drops of bromocresol green indicator solution to the Sample #1 solution.

4. Titrate with 0.1N methanolic KOH until a blue endpoint appears and persists for 30 seconds. Record the volume of titrant used to reach this endpoint as EP1.

5. Add a few drops of phenolphthalein indicator solution to the Sample #2 solution.

6. Titrate with 0.1N methanolic KOH until a faint, pink endpoint appears and persists for 30 seconds. Record the volume of titrant used to reach this endpoint as EP2.

7. Add 2 drops of saturated calcium chloride to the sample solution (**Remark 3**).

8. Titrate with 0.1N methanolic KOH until a faint, pink endpoint appears and persists for 30 seconds. Record the volume of titrant used to reach this endpoint as EP3.

9. Using Equations 2, 3 and 4 in the Calculations section of this method, determine AV-1, AV-2 and AV-3. Using Equations 5, 6 and 7 in the Calculations section of this method, determine the Monoester, Diester and Free Phosphoric Acid content.

Calculations:

Equation 1

$$\text{Sample wt.} = \frac{56.1}{(\text{Acid Value})}$$

Equation 2

$$\text{Acid Value \#1} = \frac{(EP1)(N)(56.1)}{\text{Sample wt.}}$$

Equation 3

$$\text{Acid Value \#2} = \frac{(EP2)(N)(56.1)}{\text{Sample wt.}}$$

Equation 4

$$\text{Acid Value \#3} = \frac{(EP3)(N)(56.1)}{\text{Sample wt.}}$$

Equation 5

$$\% \text{ Monoester} = \frac{[(2)(AV2) - AV1 - AV3]\ (\text{Monoester Mwt.})}{(56.1)(10)}$$

Equation 6

$$\% \text{ Diester} = \frac{[(2)(AV1) - AV2)]\ (\text{Diester Mwt.})}{(56.1)(10)}$$

Equation 7

$$\% \text{ Free } H_3PO_4 = \frac{(AV3 - AV2)\ (97.97)}{(56.1)(10)}$$

Safety: Isopropyl alcohol is flammable and a dangerous fire risk. Only handle in well ventilated areas. Potassium hydroxide is corrosive. Do not get dilute solutions in eyes, on skin, or on clothing.

Remarks:

1. The inflection points are the "true" endpoints. Therefore, the potentiometric procedure should be used whenever possible. The colorimetric endpoints do not exactly agree with the inflection endpoints. Therefore, the colorimetric procedure should only be used as a back-up procedure in case the autotitrator is not functioning properly.

2. The first endpoint typically occurs at a pH of 5.0-6.5. The second and third endpoints typically occurs at a pH of 9.5-11.0. These pH's can vary and should only be used as a guide.

3. Unless the free phosphoric acid level is very high, 2 drops of saturated calcium chloride should be sufficient. However, in samples where higher free phosphoric acid levels are expected, larger quantities of saturated calcium chloride should be used. Solubility problems may arise when adding greater amounts of calcium chloride.

ANALYTICAL METHOD

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M-015 -- Glycerin Determination (USP)

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Scope: This method is applicable to the assaying of USP grade glycerin.

Summary: The sodium metaperiodate oxidizes all glycerin present in the sample to form 2 moles of formaldehyde and 1 mole of formic acid per mole of glycerin. The formic acid is neutralized to a pH of 8.1±0.1 using 2.0N sodium hydroxide. The glycerin content is calculated after determining the volume of titrant needed to react with all formic acid present.

Apparatus:

1. Analytical balance, capable of determining weights to four decimal places.
2. pH meter capable of ±0.05 pH unit readings.
3. Electrode, combination (**Remark 1**).
4. Burette, 50 mL class A with 0.2 mL divisions.
5. Beakers, 600 mL.
6. Volumetric flasks, 100 and 1000 mL.
7. Pipettes, 1, 10, and 50 mL, class A volumetric.
8. Reagent bottle with ground glass stopper, 1 liter amber.
9. Syringes, disposable 3cc plastic.
10. Watch glasses.
11. Timer.
12. Stir plate.
13. Stir bars.

Reagents:

1. Sodium metaperiodate, reagent grade.
2. Sulfuric acid (0.1N), concentrated (80%).
3. Sodium hydroxide (0.05N), prepared and standardized using LTC-0010.
4. Ethylene glycol, reagent grade.
5. Glycerin, 99.5%+ purity.

Procedure:

Preparation of Reagent Solutions:

Sodium Periodate Solution
Dissolve 60 grams of sodium metaperiodate in sufficient water containing 120 mL of 0.1 N sulfuric acid to make 1000 mL of solution.

0.1N Sulfuric Acid Solution
Weigh about 5.15 grams of 80% sulfuric acid into a 1000 mL volumetric flask. Dilute to volume with deionized water and mix well. Label accordingly.

Ethylene Glycol Solution
Pipet 50 mL of ethylene glycol into a 100 mL volumetric flask. Dilute to volume with deionized water.

Bromothymol Blue Indicator Solution
Weigh 0.1 grams of bromothymol blue into a 100 mL volumetric flask. Pipet 1 mL of ethanol into the flask and dilute to volume with deionized water. Mix well and label accordingly.

Sample Preparation and Analysis:

1. Weigh about 0.4 grams of sample into a 600 mL beaker. Record the weight and label accordingly.

2. Add 50 mL of deionized water and 1 mL bromothymol blue indicator solution to the beaker. Add a stir bar to the beaker and mix well.

3. Acidify with 0.1N H_2SO_4 to a green or greenish yellow color.

4. Neutralize with 0.1N NaOH to a definite blue endpoint (no green).

5. Prepare a blank by adding 50 mL deionized water and 1 mL of bromothymol blue to a 600 mL beaker. After adding a stir bar and mixing well, acidify and neutralize (Steps 3 & 4) the blank solution. Label the flask as "Blank".

6. Pipet 50 mL of the Sodium Periodate solution into each of the beakers. Swirl gently. Cover each beaker with a watch glass and allow to stand in the dark for 30 minutes.

7. Pipet 10 mL of the ethylene glycol solution to each of the beakers. Swirl gently. Allow to stand for 20 minutes.

8. Add 150 mL of deionized water to each beaker and mix well. Titrate each blank and sample solution with 2.0N sodium hydroxide. The "Blank" endpoint is reached when the pH reaches 6.5 ± 0.1. The "Sample" endpoint is reached when the pH reaches 8.1 ± 0.1.

9. Using Equation 1 in the Calculations section of this method, determine the % glycerin content to one decimal place accuracy.

Calculations:

Equation 1

$$\% \text{ Glycerin} = \frac{(\text{mL Sample} - \text{mL Blank})(N)(92.10)(100)}{(\text{Sample wt.})(1000)}$$

Precision: Initial studies indicate that the relative standard deviation of the glycerin determination is $\pm 0.6\%$.

Safety: Sodium metaperiodate is an oxidizer. Use caution when handling and storing. Do not get in eyes, on skin, or on clothing.

Ethylene glycol is an eye irritant. Do not get in eyes, on skin, or on clothing. Use in well ventilated area.

Sodium hydroxide is corrosive. Do not get dilute solutions in eyes, on skin, or on clothing.

Sulfuric acid is extremely corrosive. Do not get dilute solutions in eyes, on skin, or on clothing.

Remarks: 1. Follow the care and maintenance procedures outlined by the manufacturer of the electrode.

References:

1. U.S. Pharmacopeia and National Formulary, 1995, USP 23 & NF 18, Glycerin Official Monograph, pages 713-714, 2056, and 2057.

ANALYTICAL METHOD

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M-016-- Anionic Actives, %

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Scope: This method determines the anionic active content (of known molecular weight) of synthetic detergents (i.e. DOSS-70, DTSS, and DHSS).

Summary: A hyamine titration is performed, in a stoppered graduated cylinder, to a methylene blue endpoint. The endpoint is reached when the two layers in the graduated cylinder have the same intensity of blue. If the molecular weight of the anionic active is known, the % anionic active can be calculated.

Apparatus:
1. Graduated cylinder, 100 mL with stopper.
2. Analytical balance, capable of determining weights to four decimal place accuracy.
3. Pipette, 10 mL class A volumetric.
4. Volumetric flask, 100 mL.

Reagents:
1. Chloroform, reagent grade.
2. Methylene blue indicator solution (**Remark 1**).
3. ~0.0033N Hyamine solution (**Remark 2**).
4. ~0.007N Sodium lauryl sulfate solution (**Remark 3**).
5. Concentrated sulfuric acid, reagent grade.
6. Isopropyl alcohol (IPA), reagent grade.

Procedure:

1. Using Table I as a guide, weigh an appropriate amount of sample into a 100 mL volumetric flask (**Remark 4**).

2. Add some deionized water to the flask and dissolve the sample. If the sample does not fully dissolve in deionized water alone, add sufficient IPA to fully dissolve the sample. Dilute to 100 mL with deionized water.

3. Pipette 10 mL of the sample solution into a 100 mL graduated cylinder with stopper. Add 25 mL of chloroform and 15 mL of methylene blue indicator solution to the graduated cylinder. Stopper the cylinder and shake well.

4. Titrate the solution in the graduated cylinder with standardized hyamine solution, beginning with 5 mL additions and lowering to 0.5 and 0.1 mL additions as the endpoint approaches. After each addition, stopper the cylinder, shake well for 1 minute, and allow the two layers to separate completely (**Remark 5**).

5. The endpoint has been reached when the intensity of the blue color is the same in the top and bottom layers after the two layers have separated completely. Record the titrant volume required to reach the endpoint.

6. Using Equation 1 in the Calculations section of this method, determine the % anionic actives of the sample analyzed. Report the results to one decimal place accuracy.

Calculations: Equation 1

$$\% \text{ Anionic Active} = \frac{(\text{mL Hyamine})(\text{N Hyamine})(\text{Anionic Mwt})}{\text{---------}}$$

grams of sample

Safety: Chloroform is toxic by ingestion, inhalation, and skin absorption. Use in well ventilated area and avoid getting on skin, in eyes, or on clothing.

Sulfuric acid is corrosive and can burn. Use caution when handling and avoid getting on skin, in eyes, or on clothing.

IPA is flammable. Use in a well ventilated area and avoid getting in eyes, on skin, or on clothing.

Remarks: 1. The methylene blue indicator solution is prepared by adding 0.03 grams methylene blue, 250 mL deionized water, 12 grams concentrated sulfuric acid, and 50 grams sodium sulfate into a 1000 mL volumetric flask. Mix well until all components are dissolved and dilute to volume with deionized water.

2. The 0.0033N hyamine solution is prepared by weighing 1.550 grams Hyamine 1622 (Mwt =466) into a beaker. Dissolve the Hyamine 1622 in about 100 mL of deionized water. Quantitatively transfer the solution to a 1000 mL volumetric flask. Dilute to volume and mix well. Standardize the solution against 0.007N sodium lauryl sulfate (SLS).

3. The 0.007N sodium lauryl sulfate is prepared by weighing 2 grams of Stepanol ME dry AW (Stepanol WA-100). Record the weight to nearest 0.0001 gram. Dissolve the weighed sample in deionized water. Quantitatively transfer the solution to a 1000 mL volumetric flask. Dilute to volume and mix well. Calculate the normality using the following equation: N=(grams sample x 0.995)/288.

4. If the anionic active is dissolved in a volatile solvent, weigh the sample by difference using a dropping bottle.

5. Placing the stoppered graduated cylinder on its side will decrease the amount of time required to separate the two layers.

References: 1. Toilet Goods Association, Method 110.

Table I

Molecular Weight of Anionic Active	Sample Size in grams (100% basis)
200	0.133
250	0.165
300	0.200
350	0.230
400	0.265
450	0.300
500	0.330

The molecular weights of various products are as follows:

DOSS-70	444
DTSS	749
DHSS	388

ANALYTICAL METHOD

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M-017 -- Sulfite, %

==========

Scope: This test determines the free sulfite content, as Na_2SO_3, in synthetic detergents. It is applicable to Sole-Terge 8.

Summary: An excess of standard iodine solution is added to an aqueous solution of the sample. The amount of unconsumed iodine solution is back titrated with standardized sodium thiosulfate solution. The free sulfite content is calculated from the amount of iodine consumed.

Apparatus:
1. Erlenmeyer flask, 250 mL (**Remark 1**).
2. Pipette, 10 mL class A volumetric.
3. Analytical balance, capable of measuring weight to 3 decimal place accuracy.

Reagents:
1. Iodine solution, 0.1 N.
2. Sodium thiosulfate solution (0.1N), standardized using LTC-0050.
3. Starch indicator solution, prepared using LTC-0140.
4. Glacial acetic acid, reagent grade.

Procedure:

1. Weigh approximately 2 grams of sample into a 50 mL Erlenmeyer flask. Record the weight.

2. Add 50 mL of deionized water and 5 mL glacial acetic acid to the flask. Mix well until sample is completely dissolved.

3. Pipette 10 mL of 0.1N Iodine solution into the flask. Mix well.

4. Using 0.1N sodium thiosulfate solution, titrate the sample solution to a pale yellow endpoint. Add about 2 mL of starch indicator solution to the flask and continue titrating until the blue color just disappears (usually a white endpoint).

5. Following Steps 2-4, run a blank which contains no sample.

6. Using Equation 1 in the Calculations section of this method, calculate the free sulfite content as Na_2SO_3. Report the results to one decimal place accuracy.

Calculations: Equation 1

$$\% Na_2SO_3 = \frac{(mL\ Blank - mL\ Sample)(N)(6.3)}{Sample\ wt.}$$

Safety: Acetic acid is corrosive and toxic. Use caution when handling. Do not get in eyes, on skin, or on clothing.

Iodine solution is harmful to organs, especially the thyroid. Do not get in eyes, on skin, or on clothing.

Remarks: 1. All Erlenmeyer flasks must be clean and completely dry.

ANALYTICAL METHOD

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M-018-- Solubility

==========

Scope: This method is applicable to all products.

Summary: The sample is dissolved in a specified amount of solvent. The solubility of the sample in that solvent is determined by the clarity of the solution.

Apparatus:
1. Beakers, 150 mL.
2. Stir bars.
3. Stir/hot plate.

Reagents:
1. Xylenes, reagent grade.
2. Chloroform, reagent grade.
3. Mineral seal oil, technical grade.
4. Ditridecyl adipate, technical grade.

Procedure:

1. Add sample and the appropriate solvent into a beaker in the proportions stated in the specification (**Remark 1**). Add a stir bar and mix well.

2. Allow the sample solution to settle till all air bubbles are removed. When a sample is completely soluble in the solvent tested, a clear solution results. Report the sample as soluble or insoluble.

Calculations: None required.

Precision:

Safety: Chloroform is a known carcinogen. Use in a well ventilated area. Do not get in eyes, on skin, or on clothing.

Xylenes are flammable and the vapors can be narcotic at extreme concentrations. Use in a well ventilated area. Do not get in eyes, on skin, or on clothing.

Remarks:

1. If no proportion is stated in the specifications for the product to be analyzed, prepare a 5% solution.

ANALYTICAL METHOD

M-019 – Cationic Actives, %

==========

Scope: This method is used to determine the % Cationic Active in cationic quaternary compounds with known molecular weight using titration with an anionic solution.

Summary: Using an anionic solution, sodium lauryl sulfate and cationic quaternary compounds' different color concentrations of purple in the solvent and aqueous phase, calculate the % Cationic Active of sample from the calculation section of this method.

Apparatus:
1. Graduated cylinder, 100 mL with glass stopper.
2. Analytical balance capable of determining weight to four decimal places.
3. Volumetric pipette, 10 mL.
4. Volumetric flask, 100 mL.

Reagents:
1. Chloroform, reagent grade.
2. Isopropyl alcohol, dry and neutralized.
3. Sodium lauryl sulfate (SLS) prepared by weighing out 2.88 grams Stepanol ME Dry AW and diluting it to 1000 mL with deionized water.
4. Salt buffer (50 g NaCl in 500 mL deionized water).
5. Bromophenol blue indicator solution, 0.1% in ethanol.

Procedure:
1. Weigh approximately 1-1.5 grams of sample into a 100 mL volumetric flask. Record weight.
2. Add 21 mL isopropanol and dilute to volume with chloroform.
3. Pipette 10 mL aliquot into stoppered graduated cylinder.
4. Add 20 mL chloroform, 25 mL salt buffer and 1 mL bromophenol blue indicator.
5. Titrate with 0.008N sodium lauryl sulfate solution in 1 mL increments, shaking vigorously after each addition.
6. When the emulsion breaks more readily, add 0.1 mL increments, shaking vigorously after each addition.
7. The endpoint is reached when the solvent phase (bottom layer) is very light purple and the aqueous phase (top layer) is a bright purple.

   Record the titration volume.
   Calculate % Cationic Actives using Equation 1.

Calculations: Equation 1

$$\% \text{ Active, cationic} = \frac{(\text{mL SLS})(\text{N SLS})(10)(\text{mol. wt})(100)}{(\text{sample wt})(1000)}$$

using the following average molecular weights:

CQ2: 408
CQ9: 388.6
CQ14: 335
Sanitrol: 335
Declor Aid: 335

Safety: Chloroform is toxic by ingestion, inhalation and skin absorption. Be cautious while handling it in the hood.

References: 1. Toilet Goods Association, Method 110.

ANALYTICAL METHODOLOGY

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M-020 -- Infrared Analysis

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Scope: This method is applicable to all products which are liquid, semisolid, or solid.

Summary: An infrared absorbance spectra of the sample is obtained and compared to an absorbance spectra of standard material.

Apparatus:
1. Infrared spectrophotometer, continuous scan or fourier transform, which is capable of obtaining absorbance spectra.
2. Salt plates, KBr.
3. Pellet press.
4. Mortar and pestle.
5. Rubber policeman.
6. Kimwipes.

Reagents:
1. Potassium bromide (KBr), IR grade.
2. Nujol mineral oil.
3. Methanol, reagent grade.

Procedure:

Preparation of a Liquid and Semisolid Samples:

1. Sandwich a drop of sample between two salt plates and place the salt plates in the salt plate holder (**Remark 1**).

Preparation of a Solid Sample:

1. Place about 10 mg of sample in a mortar and pestle. Ground into a fine powder.

2. Add about 300 mg of KBr to the mortar and pestle. Mix the KBr and sample well. Place enough of this mixture into the pellet press to make a KBr pellet which allows for an absorbance spectra to be taken (**See Appendix I**).

3. Place the KBr pellet on a universal sample holder.

Analysis of Sample:

1. Take a background scan of an empty sample compartment to verify that the instrument is working properly. Compare the background spectra to a standard background spectra. They should match very closely. If they do not match, any absorbance spectra obtained will not be representative of the sample.

2. If the background spectra do match, place the salt plate holder with sample (or KBr pellet) in the sample compartment. Scan the sample to obtain an absorbance spectra of the sample. Compare this spectra with a standard spectra to determine whether or not they match. Report the results as "MS" (matches standard) or "not MS" (does not match standard).

Calculations: None required.

Safety: Methanol is flammable and toxic. Use in a well ventilated area and avoid getting in eyes, on skin, or on clothing.

Remarks:
1. Clean the salt plates with an appropriate solvent (Ex. methanol) and Kimwipes before and after using. Allow the solvent to completely evaporate from the salt plates before using.

References:
1. MKP-S10 Pellet Press Instructions, Harrick Scientific Corporation.

Appendix I

Instructions for Using Pellet Press

1. Assemble both piston assemblies as follows. Select the appropriately sized die (4&7) and secure it in the end bolt (2) by tightening the set screw until snug. Back off the set screw 1/4 turn to ensure free rotation of the die.

2. Take the short neck piston assembly (4&2) and place the bolt head down.

3. Seat the collar (5) chamfered side up, on the short neck piston (4). Note: For use of 1mm or 3mm die sets, a smaller collar is used to fit within the collar shown as #5 in the assembly drawing. Both of these collars must be in place to produce small pellets.

4. Fill the collar to the brim with finely ground KBr sample mixture. The ratio of KBr to sample should be approximately 100 to 1. Using a razor blade or straight edge, scrape excess from above the rim. Collar should remain completely full.

5. Slowly screw cell body (6) onto short neck piston assembly (4). Be careful not to disturb the sample.

6. Screw the second piston assembly (2&7) onto the cell body (6) to complete construction.

7. For optimum results, evacuation should occur throughout the following procedure. Tighten the assembly with the rods provided (1), except for 10mm and 13mm pellets. For the 10mm and 13mm pellets common wrenches are all that are necessary to achieve sufficient pressures (about 40 tons per square inch) and use of the rods may lead to bent rods due to excess pressures.

8. Let press stand with pressure applied for a few minutes before disassembling.

9. Pellet may be left in the body or removed for sampling. If the pellet is to be sampled while in the cell body, the Universal Sample Holder (HUS-S1G) is suggested. However, when the pellet is removed for sampling, the Magnetic Sample Holder (HMS-S1G) is suggested. Both sample holders are available from Harrick Scientific Corporation.